Breeding of Radishes for Fusarium Resistance

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Radish yellows occurred in U.S.A. as early as 1934.[6] In Japan it was first found in 1952,[5] and prevailed over the country to establish itself as one of the most destructive diseases of Japanese radishes. The causal organism is *Fusarium oxysporum* f. sp. *conglutinance* race 2, which shows pathogenicity to a wide range of cruciferous vegetables as the race 1 (cabbage yellows pathogen) does.

The symptom begins with vascular browning, followed by yellowing and falling of leaves. Affected plants would eventually die. Since the slightest browning in the edible root greatly reduces its commercial value, very high resistance is required. But the resistance is quantitative and it is not easy to develop such strong varieties as perform well in heavily infested fields of Japan. In U.S.A. 'Red Prince' and some other varieties of highest resistance were developed.[8,10] Also several resistant varieties have been developed in Japan, but their degree of resistance and variation of variety types have not satisfied the demand.

Resistance of varieties and breeding materials[1]

1) European, Chinese and Japanese subspecies

According to Sazonova,[9] radishes as a root crop are classified into European, Chinese and Japanese subspecies based on their geographical distribution and morphological characteristics, and the former two are divided into a few groups chiefly on their earliness of maturity. While each subspecies and group include wide variation of resistance, European and Chinese subspecies are more resistant than Japanese subspecies as a whole, and the early-maturing groups of the former two subspecies show higher resistance than the respective late-maturing groups (Table 1).

2) Japanese varieties

Japanese radishes are characterized by their long, massive roots, principally related to south-Chinese radishes.[7] Following groups are important (Table 2). (1) Minowase: Heat-tolerant 'Natsu-mino' type and late-bolting 'Haru-mino' type are well developed. Fusarium-resistance is most required for the former. Higher resistance is found among 'Motohashi-' or 'Kuroba-mino' lines. (2) Nerima: Various types have long been established. 'Risou' type involving many varieties has wide variation of resistance; 'Tosai' is the strongest in this group. (3) Miyashige: Charac-

Table 1. Fusarium resistance of European, Chinese and Japanese radishes

Groups of varieties	Number of varieties[b)] Disease index[a)] 0–	20–	40–	60–
European radishes				
Late and medium maturing	4	9	2	1
Early maturing	14[c)]	10	5	1
Chinese radishes				
Late maturing	6	14	3	2
Early maturing	2	6		
Japanese radishes				
Shijuunichi group		3	2	
Minowase g.	4	13	15	
Nerima g.	2	6	5	1
Shiroagari g.	2	1	4	
Miyashige g.	2	6	5	1
Pickels-hybrids	1	1	3	
Local	1	10	10	2
Ninengo g.			4	3

a) Mean of vascular browning score, 0: all healthy–100: all killed
b) Chiefly open-pollinated varieties representative of variety groups and types
c) 'Red Prince' and 2 other varieties developed for Fusarium resistance are involved.

Table 2. Fusarium resistance of Japanese radishes

Exp. No.	Groups and types of vareties	Number of varieties[a)]			
		Disease index 20-40	40-60	60-80	80-100
I.	Minowase group				
	Minowase (M)[b)]		7	14	7
	Natsu-Minowase (M1)	1	5	5	2
	Haru-Minowase (M2)		3	9	3
	Ninengo g.				
	Mino-Toki (MT)			6	1
	Tokinashi (T)			5	
	Ninengo (N)			5	6
	Shogoin g.		1	11	4
	Shiroagari g.	1	3	7	
II.	Nerima g.				
	Akitsumari	1	1	12	1
	Ookura		3	6	1
	Miura		1	5	1
	Miyako		4	6	2
	Risou (R)	2	14	29	7
	Nerima (N)	1	8	8	3
	Takakura (T)		4	2	1
	Pickles-hybrids	2	5	11	1
III.	Miyashige g.				
	Aokubi M. Nagabuto (AN)		1	17	6
	Aokubi M. Marujiri (AM)				5
	Aokubi M. Soubuto (AS)		2	16	9
	Aokubi M. Kiributo (AK)			5	3
	Utsugi Gensuke (U)	1	1	5	
	Shirokubi M. Nagabuto (SN)			3	7
	Shirokubi M. Marujiri (SM)		4	7	1

a) F_1 hybrids or open-pollinated varieties developed before Fusarium resistant breeding was begun, including same-named ones produced by different seed growers.
b) See Fig. 1.

terized by 'Green neck', which is considered to be a trace of north-Chinese radishes. 'U. Gensuke' shows higher resistance although it is not typical of this group. (4) Shogoin: Regarded as round-rooted Miyashige. Resistance is medium to low. (5) Shiroagari: Closely related to north-Chinese radishes. Resistance is higher than the other groups. (6) Pickles-hybrids: Developed from crosses among several groups for pickles-use. A local variety 'Kotabe' is the strongest of Japanese radishes. (7) Ninengo or Tokinashi: Apparently not related Chinese radishes. Resistance is low. 'Mino-toki' varieties derived from the cross with Minowase are also susceptible.

Since radish yellows is often accompanied with virus disease, it is desirable for a breeding material to carry both kinds of resistance. Some positive correlation between both kinds of resistance had been suspected, as most of the higher Fusarium-resistant varieties were known to be virus-resistant. The relation was, however, proved to be not clear except both extremes of resistance (Fig. 1).

Varieties named samely but produced by different seed growers often show significantly different degree of resistance. As they originated from the same stocks and were not selected for Fusarium resistance, it is thought to be chiefly the result of random drift. This and crossings have probably brought much of the difference of resistance among varieties, but selection on the other traits may have also affected it indirectly (see later chapter).

Early screening techniques[2)]

1) Methods of inoculation

Yellows-resistance can be effectively evaluated by artificial inoculation at an early seedling stage. The resistance is quantitative and the amount of disease development is much affected by conditions of inoculation and environment, but the order of resistance of varieties is stable (Fig. 2). The following inoculation methods are practical.

(1) Dipping inoculation: Seedling roots are washed, then dipped in inoculum suspension and planted. Disease severity is easily controled by adjusting inoculum concentration and dipping time. It can exert higher selection pressure with less amount of inoculum, but requires more labor, than the other methods. This method is suitable for selection on population of higher resistance.

(2) Fullow inoculation: Inoculum suspension is poured in the furrow made alongside seedling rows. The merits and demerits are intermediate between dipping and soil inoculation. When seedlings are grown on rough medium, they can be simply inoculated by pouring inoculum suspension onto the medium surface, but the disease development is slow.

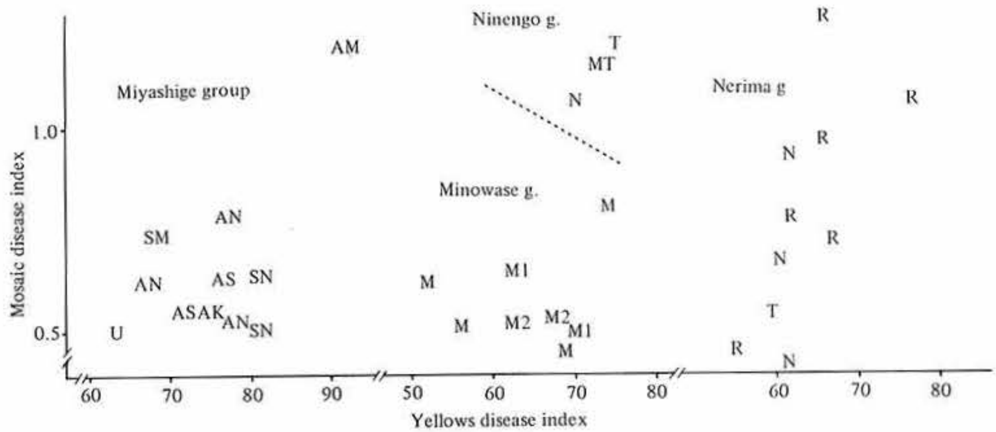


Fig. 1. Relation between mosaic and yellows resistance of Japanese radish varieties
Symbols represent means of diseases index of respective varietal types (see Table 2) or sub-types. Varieties involved are part of the varieties appeared in the figures of Table 2.

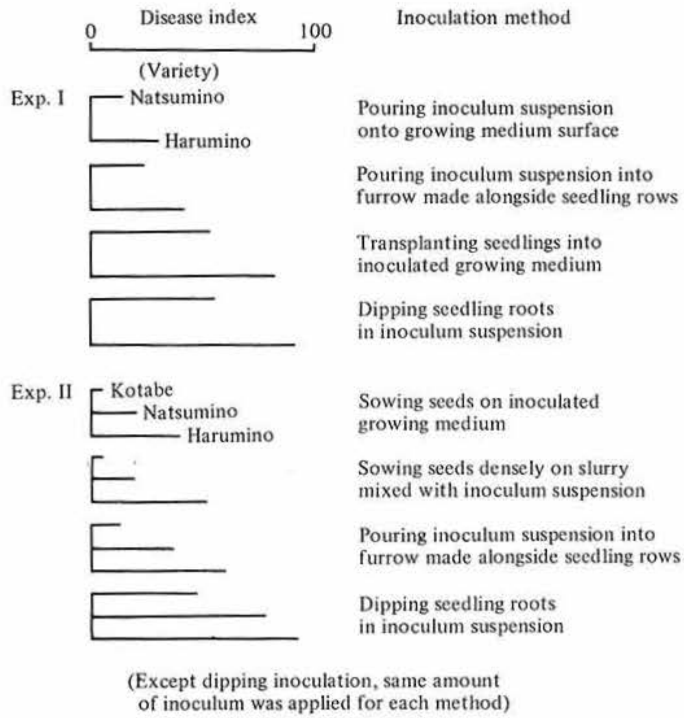


Fig. 2. Inoculation methods and radish yellows development

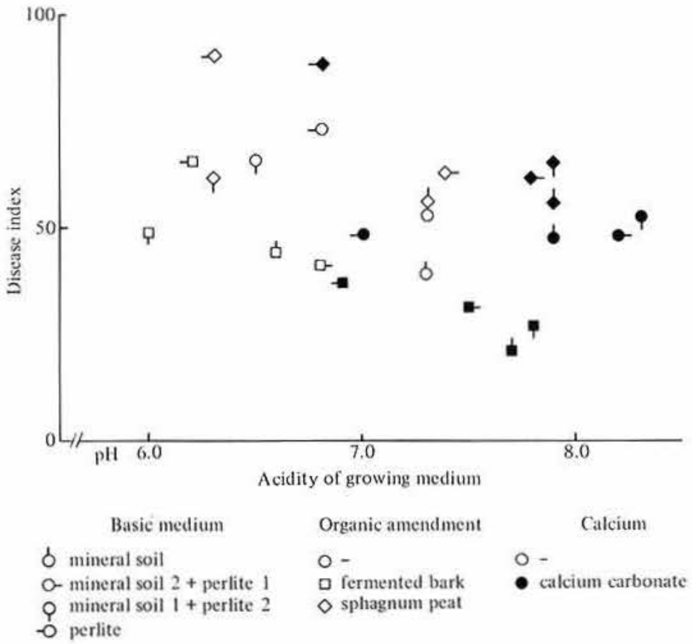
For the methods described above the advance of seedling age at the time of inoculation somewhat favors disease development, until the seedlings reach the 2-leaf-stage.

(3) Soil inoculation: Seeds are sown on inoculated growing medium. It is preferable to put disinfected medium over the inoculated one, and sow seeds on the former, so as to avoid too early death of the seedlings. Higher planting density often promotes disease development, but seedlings of obviously inferior growth due to overcrowding, low seed vigor or inadequate soil covering are apt to escape disease. Infested soil can be used repeatedly, but additional inoculation is desirable for selecting highly resistant plants. This simple method requires less labor and is precise enough so long as the growing condition is kept uniform.

(4) Slurry inoculation: Planting hole is filled with pathogen-mixed slurry, on which ten or more seeds are sown. When it is done in the fields, diseased or inferior young plants are thinned, and selected plants are grown to be examined for general characteristics. Any heavily infested medium may be used in place of the slurry, but enough moisture should be given for uniform germination and disease development. This method is suitable for a population with wide variation of resistance and general characteristics.

2) Effect of growing condition

Yellows development is facilitated by higher temperature (not beyond 28°C, optimum for screening is 22–28°C), higher soil moisture (so far as not saturated), higher soil acidity (opt. for screening is pH 6.0), N-fertilizer (preferably calcium nitrate or urea) and microelements, Sphagnum peat, artificial growing medium such as perlite and mineral soil (Fig. 3), and somewhat suppressed by K-fertilizer, fermented bark, black volcanic soil or organic soil. Dipping inoculation is less



Combination of medium factors, e.g. -◆ shows the combination of perlite, sphagnum peat, and calcium carbonate.

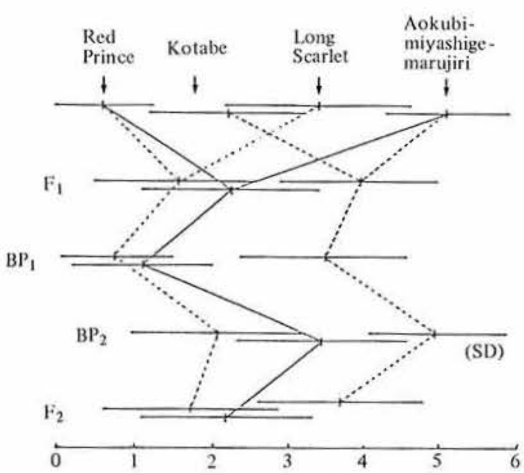
Fig. 3. Effect of composition of synthesized growing medium on radish yellows development

sensitive to environmental condition than soil inoculation.

Inheritance of resistance[3)]

By the 3 combinations of varieties differing in their degree of resistance shown in Fig. 4, each F_1 and F_2 showed intermediate disease severity between their parents, and BP_1 and BP_2, between their recurrent parent and F_1. Ratio of healthy plants varied according to the overall disease severity of the experiments. The difference of variance of disease index between F_1 and F_2 was small.

Fusarium resistance in 'Red Prince' and its relatives is reported to be polygenically controlled,[8,10)] which seems also the case of our result. But selection effect for the resistance has been so clear that several major genes may be involved.



* Based on the degree of root vascular browning and yellows symptom of leaves, 0 = healthy − 6 = killed

Fig. 4. Fusarium resistance of 4 radish varieties and their F_1, F_2 and backcross generations

The roots of F_1 between 'Red Prince' (red, small) × 'Aokubi Miyashige Marujiri' (white, large) were all purple, and the color was segregated into red, purple and white (Table 3). In the segregating generation, several quantitative traits (root length, number of leaf robes, pithyness, etc.) of the red individuals were more alike to those of 'Red Prince', while those of the white, to 'A. M. Marujiri'. Similarly, resistance was somewhat stronger in the order of the red > the purple > the white. As the root color is reported to be governed by the 2 complimentary genes,[4)] those characteristics including Fusarium resistance are considered to be partly related to the 2 genes.

Table 3. Relation between root color and Fusarium resistance in the F_2 of 'Red Prince' × 'A. M. Marujiri' grown in the infested field

Number of plant	Root color			
	White	Purple	Red	
Survived	79	207	81	
expected[a)]	92	206	69	$\chi^2=6.0$
Among survived plants				
Healthy	40	120	64	
Diseased	39	87	17	
expected	30	81	32	$\chi^2=14.8^{**}$

a) Expected ratio is 4 : 9 : 3, supposing 2 complimentary genes.

Developing Fusarium-resistant breeding stocks

Selection for Fusarium resistance has been undertaken in order to examine selection effect on several varieties as well as to develop resistant breeding stocks. Selection for resistance was done chiefly through early screening; inferior mature plants were eliminated in the fields, but variation of general characteristics was kept in the population so as to facilitate the utilization in commercial breeding program.

Selection effect was clearly seen except when disease incidence was insufficient. Although the degree of resistance had been different among the original materials, almost equally highly resistant lines have been obtained from each material after 4 to 7 times of selection (Table 4 and Fig. 5). But their resistance is still segregating, and the variation of general characteristics has been rather increased.

Table 4. Fusarium resistance of the breeding stocks

Breeding stock	Disease index	Commercial varieties	Disease index
Ano No. 1[a]	4	Natsutomi[b]	19
Ano No. 2	7	YR Kurama[c]	23
Ano No. 3	5	Hutomiya[d]	64
Ano No. 4	9	Haru-Mino	86
		Wakakoma	91

a) No. 1 to 4 was derived from 'Kotabe, Motohashi Minowase, Utsugi Gensuke, U. Gensuke × Soubutori Miyashige', respectively.
b) Highest resistant among commercial varieties
c) A resistant variety released latest
d) A medium resistant variety, developed for Fusarium resistance

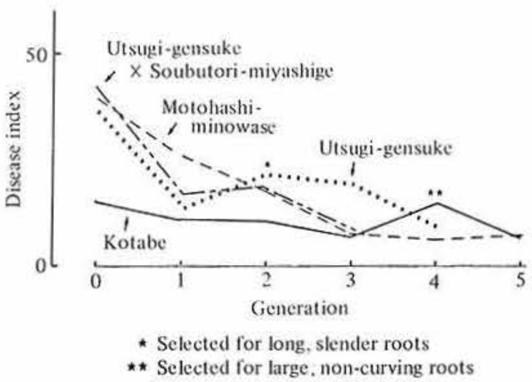


Fig. 5. Effect of selection for radish-yellows resistance

During the course of selection, 'Kotabe' was once selected for vigorous, erect roots and 'Utsugi Gensuke' was selected for slender roots, but after the selection they became less resistant. It indicates the possibility that selection for the other traits may indirectly affect resistance in some materials. For maintenance and propagation of resistant lines, stock plants should be always tested for resistance.

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